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<u>L5</u>	L3 same (advantag\$ or useful\$)	28	<u>L5</u>
<u>L4</u>	L3 same vessel	5	<u>L4</u>
<u>L3</u>	L1 same magnet\$	241	<u>L3</u>
<u>L2</u>	L1 same magnet\$ same lys\$	0	<u>L2</u>
<u>L1</u>	(extract\$ or isolat\$ or purif\$) same RNA	18802	<u>L1</u>

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<u>L2</u>	L1 same (eliminat\$ or reduc\$)	4	<u>L2</u>
<u>L1</u>	micrometal	46	<u>L1</u>

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L5: Entry 10 of 28

File: USPT

Aug 31, 1999

DOCUMENT-IDENTIFIER: US 5945525 A

TITLE: Method for isolating nucleic acids using silica-coated magnetic particles

Detailed Description Paragraph Right (27):

The method for isolating the nucleic acid using the magnetic silica particle of the present invention is capable of non-specifically adsorbing a large amount of nucleic acids and therefore has an excellent collection efficiency. Furthermore, since the method of the present invention has excellent operability, it is useful for research or the pre-treatment of clinical specimen wherein a large number of specimens must be treated in a short time. Since the method of the present invention is also capable of effectively extracting DNA and/or RNA, it is useful for a pre-treatment of various methods of amplifying a nucleic acid. In addition, since the method of the present invention separates the magnetic particle from the sample solution using a magnetic field, it can be easily automatized.

WEST

L5: Entry 13 of 28

File: USPT

Jun 16, 1998

DOCUMENT-IDENTIFIER: US 5766888 A

TITLE: Detection of carcinoma metastases by nucleic acid amplification

Detailed Description Paragraph Right (60):

In another aspect of the present invention, carcinoma metastases in selected body tissues and fluids are detected by combining the advantages of immunocytology and nucleic acid amplification technologies. In this approach, monoclonal antibodies specific for carcinoma antigens that are not expressed by the malignant cells normally present in the selected body tissue or fluid are attached to immunomagnetic beads by standard methods (Lea et al., 1986, Scand. J. Immunol. 23:509, and Lea et al., 1988, J. Mol. Recogn. 1:9). The immunomagnetic beads are then incubated with cells obtained from the selected body tissue or fluid samples. Standard magnetic separations techniques as described in Lea et al., 1986, and 1989 supra., are used to enrich the sample for carcinoma cells expressing the target antigens. The separated magnetic beads and attached carcinoma cells are placed in appropriate extraction buffers to isolate RNA. The extracted nucleic acids are then utilized in appropriate nucleic acid amplification assays to detect carcinoma associated mRNA.

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L5: Entry 19 of 28

File: USPT

Sep 9, 1997

DOCUMENT-IDENTIFIER: US 5665582 A
TITLE: Isolation of biological materials

Detailed Description Paragraph Right (62):

Magnetic cell separations have been achieved for several years, however, investigations using magnetic particles for immunoassays, isolation of RNA, DNA, viruses and subcellular compartments have been initiated only recently because magnetic particles with the appropriate properties necessary for various applications have been developed only in the last few years (for review, see Haukanes and Kvam, 1993). The use of monodispersed superparamagnetic microparticle preparations (sized in the micron range) coupled to monoclonal antibodies specific to cell surface proteins was pioneered by Treleaven et al. (1984) for affinity separation of neuroblastoma cells from bone marrow. These types of particles have since proven useful for other cell separations by positive enrichment, including incomplete removal of lymphocyte subsets for quantification (Brinchmann et al., 1988), and isolation of lymphoid cells from peripheral blood (Vartdal et al., 1986). Magnetic microparticles have been successfully used for recovery and genetic analysis of specific nucleic acids (Albretsen, 1990), and isolation of DNA binding proteins (Grabrielsen et al., 1990). The present inventors have determined that these particles are inadequate for plastid isolation from whole cell lysates owing to the nonspecific reactivity of the particle surface coatings or the relative size of the particles. However, plastids can be immobilized on these particles to facilitate handling when used in biochemical assays which require several incubation or wash steps. Immobilization of prefractionated stacked golgi to magnetic microparticles coupled to Sec 7p antibodies has proven useful for cell-free analysis of membrane traffic in transport vesicles (Franzusoff et al., 1992).

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L1 same nucleic

1

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<u>L1</u>	shak\$ same lys\$ same cell\$	10	<u>L1</u>

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L2: Entry 1 of 1

File: USPT

Oct 20, 1992

DOCUMENT-IDENTIFIER: US 5157106 A

TITLE: N-terminal deletions of lymphotoxin, their preparation and use

Detailed Description Paragraph Right (11):

20 g of E. coli cells which had been spun down from appropriate shake cultures (see Examples 2+6) were taken up in 200 ml of 20 mM sodium phosphate buffer, pH 8.5, and the mixture was treated with ultrasound for 15 minutes and then 4 ml of 2 M MnCl₂ solution were added to precipitate the nucleic acids. After centrifugation, the supernatant was adjusted to pH 8.9 with dilute ammonia solution, and the crude protein was precipitated with 78 g of ammonium sulfate. The precipitate was centrifuged and isolated, dissolved in 100 ml of 10 mM sodium phosphate buffer, pH 9.0, and was dialyzed against this buffer. Chromatography on Q-Sepharose and S-Sepharose (supplied by Pharmacia) resulted in a homogeneous protein solution which contained delta 23 lymphotoxin with methionine at the N-terminal end and in a purity exceeding 99% (according to SDS gel electrophoresis analysis). The protein had the N-terminal sequence Met-His-Ser-Thr-Leu-Lys-Pro-Ala-Ala-His-Leu-Ile.

Detailed Description Paragraph Right (13):

176 g of wet biomass from E. coli shake cultures producing delta 25 lymphotoxin (see Examples 4+6) were, after centrifugation, suspended in 600 ml of buffer (20 mM sodium phosphate, 400 mM arginine, pH 8.5). The cells were disrupted by treatment with ultrasound at 4.degree. C. The suspension was adjusted with 2 M MnCl₂ solution to a final content of 40 mM to precipitate the nucleic acids. At the same time, the pH was adjusted to 7.5 with 12.5% ammonia solution. After centrifugation, 390 g of ammonium sulfate per liter of supernatant were added at 4.degree. C. to precipitate the delta 25 lymphotoxin. The precipitate was dissolved in 300 ml of 20 mM sodium phosphate buffer, pH 10.5. The cloudy solution was centrifuged to remove insoluble protein aggregates. The supernatant was dialyzed against 5 mM sodium phosphate buffer, pH 8.5, and then chromatographed on a Q-Sepharose column (supplied by Pharmacia). For final purification, the fraction containing delta 25 lymphotoxin was subjected to successive chromatography on CM-Sepharose and Q-Sepharose (both supplied by Pharmacia). SDS polyacrylamide gel electrophoresis showed that the purity of the resulting protein was greater than 99%. Depending on the fermentation conditions, the protein contains little or no N-terminal methionine, and the N-terminal sequence was determined to be Thr-Leu-Lys-Pro-Ala-Ala-His-Leu-Ile-Gly.

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L5: Entry 25 of 28

File: USPT

Apr 30, 1996

DOCUMENT-IDENTIFIER: US 5512439 A

TITLE: Oligonucleotide-linked magnetic particles and uses thereof

Brief Summary Paragraph Right (11):

Several advantages of the use of magnetic particles stand out clearly. The magnetic particles can be added to a mixture containing the target nucleic acid, e.g. a cell extract, stirred and then magnetically drawn to one side of the receptacle. The liquid can then be removed together with unwanted components and the magnetic particles, having the RNA bound thereto, can then be redispersed in a washing solution. The washing step can be repeated several times in quick succession. The whole process of obtaining the target nucleic acid can be performed in under 15 minutes.

102(c)

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L5: Entry 24 of 28

File: USPT

Jun 4, 1996

DOCUMENT-IDENTIFIER: US 5523231 A

TITLE: Method to isolate macromolecules using magnetically attractable beads which do not specifically bind the macromolecules

Detailed Description Paragraph Right (10):

Magnetic bead induced precipitate separation has been shown to greatly improve the process of hydrateable polymer/salt precipitation of bacteriophage and other viruses. The hydrateable polymer/salt precipitation has been shown in FIG. 2. Magnetic bead induced precipitate separation has also been shown to greatly improve the precipitation of bacterial DNA, membranes and proteins from bacterial lysates containing RNA and low molecular weight DNA species. The preparation of RNA and low molecular weight DNA species as modified by this invention has been shown in FIG. 3. The combination of these two procedures can be used to derive a novel procedure for the purification of low molecular weight DNA from bacteriophage or other viral particles. In this novel procedure, particles of bacteriophage or other viruses are precipitated using the magnetic bead method given in FIG. 3. The purified particles are then subjected to lysis by sodium hydroxide and SDS. This step separates the coat proteins from the DNA, with both being released into solution. Magnetic beads and either sodium or potassium acetate are then added at low pH. This causes the SDS and coat proteins to precipitate. The precipitate also entraps the magnetic beads. A magnetic field is then applied to the precipitation. This magnetic field is used to draw the complex of magnetic beads and precipitated material to the bottom (or side) of the tube. The supernatant is then removed from the tube with a pipette whilst the complex of beads and precipitated material is held against the bottom (or side) of the tube by the magnetic field. The low molecular weight DNA can be purified and/or concentrated from this supernatant by alcohol precipitation as described above. The modification of low molecular weight DNA preparation from bacteriophage or other viruses by this invention has several clear advantages over the conventional method of precipitation using centrifugation and other methods. The procedure, as modified by this invention, is:

162(e)